A Study of the Hatching Process in Aquatic Invertebrates

IX. Hatching within the Brood Sac of the Ovoviviparous Isopod, *Cirolana* sp. (Isopoda, Cirolanidae)

X. Hatching in the Fresh-water Shrimp, *Potimirim glabra* (Kingsley) (Macrura, Atyidae)

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THROUGH the kind cooperation of the Instituto Tropical de Investigaciones Cientificas of the Universidad de El Salvador, and through a travel grant provided by the Graduate Research Fund of Western Reserve University, it was possible to continue, in the summer of 1962, a study of the hatching processes of aquatic invertebrates in the tropical environment of El Salvador, Central America. I wish to express my gratitude to the sponsoring agencies for their aid in making the study possible.

Previous publications in the present series are listed in Davis (1964). As has been shown in these publications, hatching may be achieved by the swelling of the embryo (growth or water uptake), by mechanical means, by osmosis through a nonliving egg membrane, by enzymatic action or by some combination of these methods.

IX. HATCHING WITHIN THE BROOD SAC OF THE OVOVIVIPAROUS ISOPOD, *Cirolana* sp. (ISOPODA, CIROLANIDAE)

Numerous specimens of *Cirolana* sp. were collected on July 23, 1962, from empty shipworm galleries in pieces of old wood wedged in the rocks between the tide marks near the village of Mizata, in the eastern portion of El Salvador (Departamento de La Libertad). Specimens were identified as possibly a new species of *Cirolana* by Dr. H.-E. Gruner, Zoologisches Museum, Berlin, and as being close to *C. diminuta* Menzies (but possibly as a new species) by Dr. Robert J. Menzies, Duke University Marine Laboratory, Beaufort, N.C.[2]

Living specimens were returned to the laboratory for study. Many of them contained eggs or young in their brood pouches at all stages of development. The earlier stages of embryonic development were enclosed in definite egg membranes, but later stages were free in the pouches.

In *Cirolana* sp. the brood pouch is so firmly enclosed that the eggs and young could be removed only by disruption of the body of the mother. Recently laid eggs removed in this fashion (Fig. 1) were ovoid, about 730μ long and 600μ wide. The egg membrane was not turgid, and fit rather loosely, leaving a considerable space between it and the embryo. Later, when the young isopod took on more definitive form (Fig. 2), the entire mass increased greatly in size so that, although the width decreased somewhat to 540μ, the length became 1,350μ. At this time the egg membrane had come to fit the embryo much more tightly but, as shown in the figure, small fluid-filled spaces still occurred in front and just behind the head, as well as lateral and posterior to the abdominal segments. The beating heart could be seen clearly in the region between the thorax and the abdomen. Just before hatching from the egg membrane, all fluid-filled spaces disappeared, and the embryo increased to an average of 1,590μ $\times$ 580μ (10 measurements), an increase of 2.2 times in length.

Hatching itself was passive as far as movements of the appendages of the embryo were concerned. Swelling became much more rapid, so that the length increased within 15 min

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from an average of 1,590 to 1,770μ. No signs of drinking were observed at this time, and therefore the obvious uptake of water must have been across the surface of the embryo after the water had permeated through the egg membrane. Thereupon the embryo squirmed and twisted, and the membrane broke at the anterior end, liberating some of the anterior appendages. It took approximately 1 min for the membrane to slip off, thus completing the hatching. At an average of 7 min after the break in the membrane occurred, the young isopods had increased to a length of 1,890μ.

It was observed that embryos within their membranes, whether some time before hatching, or nearly ready to hatch, were able to live for over 24 hr in the external sea water, but they became weaker and weaker, and shortly thereafter death ensued. In those ready to hatch, hatching was unsuccessful if they had been out of the brood pouch more than about an hour before initiation of eclosion. It is not thought that the sea water used was toxic, for the parents were living in it. Either lack of nutrients pro-

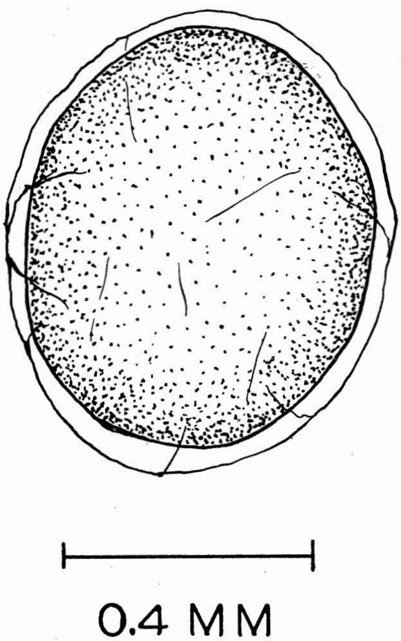

FIG. 1. *Cirolana* sp. Early egg removed from the brood pouch. There is a large fluid-filled space between the developing embryo and the loosely fitting egg membrane.

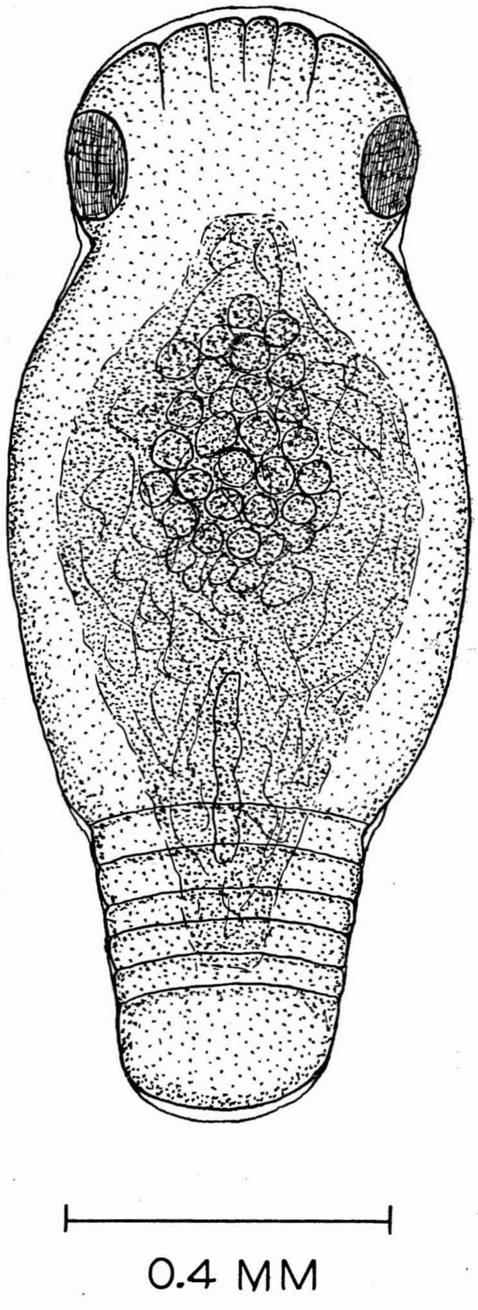

FIG. 2. *Cirolana* sp. An embryo shortly before hatching. There has been a great increase in size, compared to the early stage shown in Figure 1. The membrane is thin and tightly stretched. A fluid-filled space remains, however, between it and the embryo.

vided to the embryos within the brood pouch by the mother or lack of ventilation of the embryos may have contributed to their weakening. Secretion of nutrients by the mother has long been suspected for isopods, but no definite evidence proves its occurrence, at least prior to hatching. Wesenberg-Lund (1939) has questioned the production of such nutrient substances in fresh-water isopods. Based on length measurements of marsupial young, and on ex-marsupial cultures of embryos, Lemercier (1957) denied the existence of such secretions by the aquatic *Jaera marina.* Saudray and Lamercier (1960), on the other hand, showed that although there was a decrease of vitelline reserves (and of dry weight) before hatching in eggs of the terrestrial *Ligia oceanica,* there was a considerable increase of weight, in great part from minerals, after hatching, and that this increase probably was of maternal origin.

The newly hatched young were by no means able to care for themselves. Locomotion was impossible; although in the experimental containers they appeared to attempt to right themselves after hatching from their (invariable) position on their backs, they never succeeded in doing so. Specimens that were pushed by the investigator into a position with the ventral side down always rolled over on their backs immediately when the supports were removed. Likewise, embryos within the egg membranes were almost invariably lying dorsal side down, though a few were not.

After hatching the young remained in the brood pouch for a considerable period of time (the length of their stay could not be ascertained). During their sojourn in the brood pouch they developed a great deal more pigmentation than the small amount present at the time of hatching, and they increased considerably in size. Some attained a length of a little more than 2 mm, or one-third the length of the parent. Liberation of the young from the brood pouch was not observed.

Discussion

There was considerable increase in size of the embryo before hatching, and even more subsequently. It was not possible to distinguish between true growth (i.e., increase of biomass) and false growth by intake of water. True growth could occur only if the mother provided some nutrition to the developing young.

The extremely rapid growth occurring at the time of hatching could not have been a true growth, for new protoplasm cannot be formed so rapidly. It is believed that the size increase came about by the absorption of water from the environment, and that it probably was associated with the liberation of osmovalent substances in the protoplasm or the hemocoel at this time.

Experimental tests of the permeability of the egg membrane could not be undertaken, but the steady growth of the embryo and of the postembryonic animal, and the rapid growth at the time of hatching, indicate that the membrane was permeable both to water and to any nutrient substances the mother may have secreted into the brood pouch. Originally loosely surrounding the egg, the egg membrane later became taut and invested the embryo closely. This does not suggest that the membrane itself was osmotically active, as occurs in hatching copepods and in some insects (Davis, 1959, 1961).

Hence the hatching process appears to proceed somewhat as suggested by Przylecki (1921) for *Daphnia,* where the embryo swells (in *Daphnia* only by the uptake of water) until finally the egg membrane is sloughed off.

Hatching has previously been described briefly for isopods by Ellis (1961), who said that in *Asellus intermedius* there is an outer egg membrane, an inner egg membrane, and a larval membrane. The outer membrane splits and is shed at an early stage of development. The other two membranes are shed later. Nothing is said of an increase of embryo size, however. Earlier investigators (Verhoeff, 1920; Forsman, 1944; Naylor, 1955) mentioned an increase of size. Both Forsman and Naylor studied aquatic isopods (respectively, *Jaera albifrons* and *Idotea emarginata*). Forsman said that the first stage of life in the brood pouch terminated when the egg shell suddenly burst and slipped off. The enclosed embryo immediately increased in size but was still enclosed in an "embryonic membrane." Only after some time the latter suddenly ruptured. It is not clear from Forsman's description that the size increase caused either of the eclosions, but he specifically stated that final emergence from the second membrane was by the struggles of the animal. Naylor (op. cit.) observed that the originally spherical egg became ovoid after a period of development, and that this was followed by the rupture of the egg membrane. There was a growth from 0.7-mm diameter in the spherical egg to an embryo 1.2 mm long. This embryo was still enclosed in an "embryonic membrane." After appendages appeared in the developing embryo, this membrane also ruptured.

In the present study only a single egg membrane was observed, but the inability of the embryos to continue living normally outside of the brood pouch, combined with the rapidity of movement of the parent animals and the opaqueness of their bodies, may have led to a second membrane being overlooked. In the earliest stages observed there was no sign of more than one membrane; the fact that this one membrane lay rather loosely around the embryo does not suggest that an outer membrane had been sloughed off earlier.

Summary

Eggs at various stages of development, and hatched young, were taken from the brood pouches of *Cirolana* sp. There was approximately a doubling of the size of the eggs from time of oviposition to hatching, and the young increased in size even more after hatching. It remains unclear whether the mother secretes a nutritive substance into the brood pouch. Hatching itself was accompanied by, and partly caused by a sudden and rapid increase of volume of the embryo, evidently through a rapid uptake of water across the body surface. The stretched egg membrane, however, was burst by squirming action of the embryo.

X. HATCHING IN THE FRESH-WATER SHRIMP, *Potimirim glabra* (Kingsley) (MACRURA, ATYIDAE)

Several small shrimps were obtained from their shelter beneath rocks in a small stream (Rio Chiquileco), near Mizata, Departamento de La Libertad, El Salvador on July 23, 1962. They were found not more than 20–30 m from the ocean beach, but the water in which they

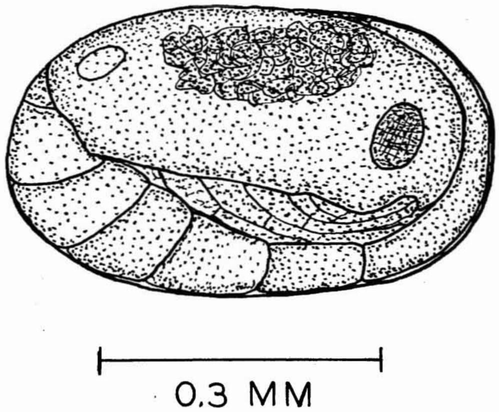

FIG. 3. *Potimirim glabra.* The young within the egg shortly before hatching. The telson passes anteriorly in front of the head, to terminate dorsally over the yolk mass.

lived was fully fresh (though probably subject to salt-water contamination during storms). One of the shrimps was ovigerous and was returned to the laboratory for closer study. The shrimp was identified as *Potimirim glabra,* and the determination has been confirmed by Prof. Dr. L. B. Holthuis of the Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands. Evidently the species has been reported only twice before, by Kingsley (1878) from the west coast of Nicaragua, and by Holthuis (1954*a*, *b*) from the Rio Jiboa in El Salvador, about 60 km east of the present find.

When the specimen was captured the eggs were at a very early stage of development, but the shrimp and its eggs lived well in fresh water in the laboratory. Five days later the eggs were far advanced, for the eyes were conspicuous, the hearts were beating (the rate of the heart beat varied in the different specimens), and there were vigorous intestinal movements. Occasionally there was a sudden twitch of the body. The average size of seven eggs was $500\mu \times 300\mu$ (they varied little in their dimensions).

Within the egg (Fig. 3) the lengthy young were coiled into an oval. The first two abdominal segments lay more or less along the same axis as the cephalothorax, but the rest of the abdomen folded under so that most of the segments lay ventral to the cephalothorax and its appendages. The furca was folded over the head, terminating dorsally. The furcal setae passed back to the region between or just posterior to the eyes (hence over the region of the anterior part of the yolk mass).

Because of the proximity of the habitat to the sea, it was uncertain whether hatching occurred in fresh water or in sea water. Some of the eggs were placed in sea water, but after a couple of hours they showed distinct signs of degeneration. Controls in fresh water remained normal.

Hatching commenced on the sixth day after capture of the animal. The size of the eggs had increased slightly to an average of $520\mu \times 310\mu$. At this time some more eggs were placed in sea water; they did not hatch successfully, whereas nearly all of the controls in fresh water did so.

Hatching was observed many times. Two membranes surrounded the egg (a third membrane, as described by Burkenroad, 1947, was not observed). Before hatching, the embryo lay snug within the two membranes, but it was able to move around in such a fashion that it was clear the embryo itself was not exerting pressure on the outer membrane.

The first event of hatching was the sudden bursting of the outer membrane, always at the cephalic end of the embryo. Immediately afterwards the inner contents, surrounded by a second membrane, almost explosively increased in size, showing that they were under considerable internal pressure. At this time the dimensions of the embryo and its membrane were about $600\mu \times 320\mu$. Soon there was a rather rapid enlargement to $650\mu \times 330\mu$. As this swelling occurred, the outer membrane slipped to the posterior end of the egg and was sloughed off.

At first after the bursting of the outer membrane the inner membrane lay tightly appressed against the body of the embryo, but later a fairly large space developed between the living animal and the nonliving membrane. At the same time the folds of the animal loosened up somewhat within the membrane, so that spaces appeared ventrally between the thorax and the abdomen.

Then the young animal commenced to struggle, primarily by movements of the abdomen. Eventually the inner membrane broke over the end of the telson, hence over the head (no egg burster was evident on the telson), and the animal straightened out. In this manner the membrane was torn still more. Then a single vigorous flip was sufficient to liberate the zoea from the remains of the egg membrane.

Five specimens were timed in their emergence. They took from 2 min 15 sec to 17 min 5 sec (average of 6 min 35 sec) from the first bursting of the outer membrane to the last flip from the inner membrane.

Discussion

Hatching in decapod crustaceans has seldom been described heretofore. Among publications touching on the process, Davis (1959) sketchily observed hatching in the Ohio fresh-water shrimp, *Palaemonetes kadiakensis,* and stated that osmotic forces were involved. R. L. Robertson, writing in Truitt (1941:10), gave a figure showing hatching of an egg of the crab, *Callinectes sapidus.* The prezoea is shown escaping backwards, with the rear of the head coming out first; only an outer membrane is shown. Churchill (1917–1918) stated concerning the same species: ". . . the shell of the egg split into two parts, the young crab emerged and, after freeing itself from a thin membrane which covered it, swam away." Gray (1942) briefly described hatching of the fiddler crab, *Uca minax.* She intimated it was caused entirely through the vigorous mechanical action of the crab; only one egg membrane was mentioned. Andrews (1904, 1907) studied hatching in the crayfishes *Astacus leniusculus* and *Cambarus affinis,* but merely stated that: "In hatching, the egg capsule burst open over the back of the embryo, and . . . then the embryo slowly glided out backward." According to his observations the embryo at hatching was very inactive and helpless, unable to use its limbs. More recently, Burkenroad (1947) described hatching in the marine shrimp *Palaemonetes vulgaris* and in its close relative from fresh water, *P. exilipes.* In the marine species, the outer two membranes were burst by swelling of the young within, and the embryo, enclosed in the third membrane, emerged passively. It then tore its way from the third membrane by active extension of the pleon. In the fresh-water form, on the other hand, the outer membranes were split through the pressure caused by an osmotic swelling of the inner membrane. The swelling continued until the outer membranes were sloughed off and until the inner membrane burst and liberated the young. Herrick (1911) has described some aspects of hatching in the American lobster, *Homarus americanus.* He observed that an outer membrane burst first, and that an inner membrane, which was attached to the outer one, remained surrounding the hatching larva. When the inner membrane was shed the larva emerged, but must then molt before it was free-swimming.

The bursting of the outer egg membrane in *Potimirim glabra* could not have been associated with the presence of any hatching spines or egg bursters. The only structures so placed on the embryo that they might have acted in this manner were the anteriorly directed lateral spines on the carapace, but if these had caused the bursting of the outer membrane they would also have done so to the inner membrane, which remained intact. It was clear that the bursting was caused by considerable pressure from within, and it is postulated that this occurred by the osmotic uptake of water through the inner membrane, although perhaps the embryo itself took up water also, as indicated by the fact that there was no space between the embryo and the inner membrane at the time that the outer membrane broke. That such a space appeared later, however, suggests that osmotic water was being taken in through the membrane. Estimates of possible further increase in size of the embryo itself at this time, however, were not possible because of the manner in which it loosened up its compact folds within the inner membrane. This made accurate comparative measurements impracticable.

The fact that the outer membrane invariably burst over the cephalic region suggests a weakened line of dehiscence there, although before bursting no such line was visible with the high power of a compound microscope.

Hence, in *Potimirim glabra* the hatching process is a combination of osmotic bursting of an outer membrane and active mechanical exit by the animal through the inner membrane. Evidently no enzymatic action is involved, unless there is an enzymatically caused change in the permeability of the inner membrane at hatching time.

Summary

Potimirim glabra hatched through the osmotic swelling of an inner egg membrane; the pressure caused the rupture of the outer membrane, which then was sloughed off by continued swelling of the inner one. Final hatching from the inner membrane was accomplished by the struggles of the young shrimp, which broke the membrane over the head by action of its telson, then tore the membrane further by straightening out its highly coiled body.

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